New DNA Tests on Tap To I.D.

Bad Microbes

Candida albicans and *Listeria monocytogenes* are two bad microbes. The first, a yeast, can be especially lethal to individuals with weakened immune systems. The second, a foodborne bacterium, kills hundreds of people in the United States each year and is the leading cause of costly food recalls due to microbial contamination.

Now, ARS scientists in Peoria, Illinois, have devised a new DNA-based approach for identifying these pathogens that's faster, easier to use, and more precise than some currently used methods.

For example, pulsed-field gel electrophoresis (PFGE) is considered the gold standard for genetically identifying *L. monocytogenes* bacteria that cause food poisoning. But PFGE is difficult to run, takes about 3 days, and has several disadvantages that complicate efforts to determine the relationships between different isolates.

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In contrast, "Our method can be performed in a single day," says microbiologist Todd Ward, at ARS's National Center for Agricultural Utilization Research, in Peoria, "and can target single nucleotide variations within specific genes."

Such variations help distinguish one strain of *L. monocytogenes* from another. By targeting genes for virulence, for example, the test could enable a user to understand what makes some strains more harmful or better adapted to a particular environment than others.

This feature could prove especially useful in hazards analysis and critical-control-point programs at food-processing plants. "The ability to identify *Listeria* that have colonized your production plant can help determine whether food products are contaminated before coming into the plant or within the plant by resident strains," says Ward.

Brent Page, a molecular biologist and colleague in the ARS center's Microbial Genomics and Bioprocessing Research Unit, and research leader Cletus Kurtzman say the test could be used to check for yeasts that cause food and beverage spoilage. It could also speed the search for yeasts adept at fermenting cornstarch into ethanol or those used for biocontrol of fruit-storage rots.

On the medical front, the test may enable hospital clinicians to cast a broader net for the 30 to 40 *Candida* species that can cause human infections, particularly in immunocompromised people. Page says the various culture-plate testing methods now used to diagnose *Candida* infection are limited to a few species—notably *C. albicans*—and the turnaround time is 24 hours to a few weeks, delaying treatment. Genetic fingerprinting tests used in some labs may be faster, but they too detect only a few *Candida* species.

By comparison, the test from Kurtzman's unit identifies 32 total species—simultaneously and in less than 5 hours.

In a machine called a "flow cytometer," which can handle up to 100 samples at a time, molecules called "probes" find and bind to corresponding pieces of species-specific DNA. The researchers created the probes using DNA-sequence information from their unit's microbial-genomics database. The probes have a fluorescent marker that tells the cytometer which DNA sequence was detected. The machine clearly displays the species' identity as color-coded bar graphs.

Such accuracy stems from Kurtzman's and University of Miami professor Jack Fell's co-development of gene-sequence databases for all known yeast species, leading to the current effort, which was initiated with funding from the National Institutes of Health.

Commercial collaborators are being sought to develop the resulting tests in kit form.—By **Jan Suszkiw,** ARS.

This research is part of Food Safety (Animal and Plant Products), an ARS National Program (#108) described on the World Wide Web at www.nps.ars.usda.gov.

Todd J. Ward, Brent T. Page, and Cletus P. Kurtzman are with the USDA-ARS National Center for Agricultural Utilization Research, 1815 North University St., Peoria, IL 61604; phone (309) 681-6560, fax (309) 681-6672, e-mail wardtj@ncaur.usda.gov, pageb@ncaur.usda.gov, kurtzman@ncaur.usda.gov. ✸

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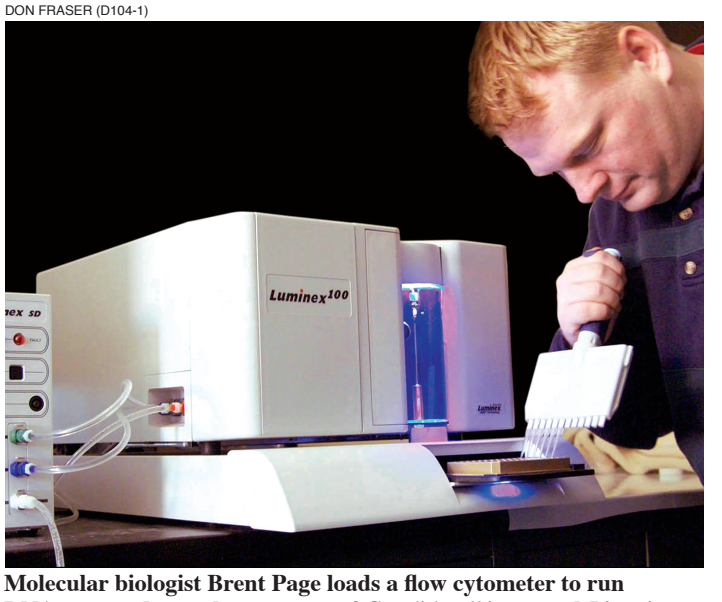

Molecular biologist Brent Page loads a flow cytometer to run DNA tests to detect the presence of *Candida albicans* and *Listeria monocytogenes*.